TOXICOLOGICAL SCIENCES 89(1), 93–107 (2006)
doi:10.1093/toxsci/kfj011
Advance Access publication October 12, 2005

Gene Expression Profiles in Rat Liver Treated With Perfluorooctanoic Acid (PFOA)

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Received July 26, 2005; accepted September 22, 2005

Perfluorooctanoic acid (PFOA; Pentadecafluorooctanoic acid) is widely used in various industrial applications. It is persistent in the environment and does not appear to undergo further degradation or transformation. PFOA is found in tissues including blood of wildlife and humans; however, the environmental fate and biological effects of PFOA remain unclear. Microarray techniques of gene expression have become a powerful approach for exploring the biological effects of chemicals. Here, the Affymetrix, Inc. rat genome 230 2.0 GeneChip was used to identify alterations in gene regulation in Sprague-Dawley rats treated with five different concentrations of PFOA. Male rats were exposed by daily gavage to 1, 3, 5, 10, or 15 mg PFOA/kg, body weight (bw)/day for 21 days and at the end of the exposure, liver was isolated and total liver RNA were used for the gene chip analysis. Over 500 genes, whose expression was significantly ($p < 0.0025$) altered by PFOA at two-fold changes compared to control, were examined. The effects were dose-dependent with exposure to 10 mg PFOA/kg, bw/day, causing alteration in expression of the greatest number of genes (over 800). Approximately 106 genes and 38 genes were consistently up- or down-regulated, respectively, in all treatment groups. The largest categories of induced genes were those involved in transport and metabolism of lipids, particularly fatty acids. Other induced genes were involved in cell communication, adhesion, growth, apoptosis, hormone regulatory pathways, proteolysis and peptidolysis and signal transduction. The genes expression of which was suppressed were related to transport of lipids, inflammation and immunity, and especially cell adhesion. Several other genes involved in apoptosis; regulation of hormones; metabolism; and G-protein coupled receptor protein signaling pathways were significantly suppressed.

Key Words: perfluorinated compounds; PFOA; GeneChip; liver gene expression; fatty acid and lipid metabolism.

Perfluorinated compounds (PFCs) have been manufactured and used for various industrial applications for over 50 years (Giesy and Kannan, 2002; Sohlenius *et al.*, 1994). For example, PFOS has been used as a surfactant and surface protector in carpet, leather, paper, and food containers; as well as performance in chemicals in products such as fire-fighting foams, floor polishes, and personal care products (OECD, 2002). The high-energy C-F covalent bonds in PFCs are strong enough to resist hydrolysis, photolysis, biodegradation, and metabolism and thus result in a high degree of environmental persistence and bioaccumulation of PFCs and their derivatives. A number of commercially used PFCs have been shown to occur in the environment, including perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorooctane-sulfonamide (PFOSA), perfluorohexanesulfonate (PFHS), perfluorobutane-sulfonate (PFBS), and perfluorononanoic acid (PFNA) (Giesy and Kannan, 2002).

The vapor pressure and solubility of PFOS (K^+) are 3.31×10^{-4} Pa and 0.5 g/l and those of PFOA (H^+) are 70 Pa and 9.5 g/l, respectively (Hekster *et al.*, 2003). Even though there are great differences in the physical and chemical properties of PFOA and PFOS, they were found to be the dominant PFCs in the environment and were found to be widespread in biota, such as wild birds (Giesy and Kannan, 2001), dolphins (Kannan *et al.*, 2001), polar bears and ringed seals (Martin *et al.*, 2002), shell and finfish (Hoff *et al.*, 2003; Van de Vijver *et al.*, 2003), and in human serum and seminal plasma (Guruge *et al.*, 2005; Olsen *et al.*, 1999). They are usually present as a dissociated anion or ion pair in aqueous media; Kannan *et al.* (2005) has shown that PFOA is less restrained in the body and less bioaccumulated among trophic levels compared with PFOS.

Previous studies have demonstrated that PFOS could affect the membrane fluidity and mitochondrial membrane potential *in vitro* and *in vivo* (Hu *et al.*, 2002, 2003). Also, PFOS inhibited gap junction intercellular communication (GJIC) in a dose-dependent manner (Hu *et al.*, 2002) and peroxisomal fatty acid β-oxidation was found to be the major pathway affected by PFOS.

Several studies have shown that PFOA has the potential to induce peroxisomal β-oxidation in the liver of male rats but not in female rats, whereas PFOA induced the activity in cultured

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patocytes from both male and female rats (Berthiaume and llace, 2002; Kudo *et al.*, 2002). Increases in β-oxidation of ty acid, several cytochrome P-450 (CYP450) mediated ctions and inhibition of the secretion of low-density lipopro-ns and cholesterol from liver have also been demonstrated ennedy *et al.*, 2004). However, the mechanisms of action and tabolic pathways affected by PFOA are still unclear.

Because, there are a number of PFCs with similar structures which organisms can be exposed simultaneously, risk assess-nt of these complex mixtures need to be considered. Toward s end, it is important to know if the various PFCs have the ential to elicit similar responses through similar mecha-ms and modes of action and whether their risks need to be essed based on similar joint action or independently. In the sent study, the effects of PFOA on liver gene expression in s was determined by use of microarray techniques to ermine effects on gene expression patterns relative to that nexposed rats. This information was then used to determine ch metabolic and signal transduction pathways were cted. This information can then be used to determine the nary pathways that are affected and to infer mechanisms of on. The results of this study can then be compared to those h other PFCs, such as PFOS (Hu *et al.*, 2005a,b).

MATERIALS AND METHODS

nimals and administration. Seven-week-old male Sprague-Dawley rats obtained from CLEA Inc. (Tokyo, Japan) and housed at 20–24°C in idity-controlled (40–60%) facilities at the National Institute of Animal th, Japan. After acclimatization to a standard diet (MF, Oriental Yeast Co., n) for 1 week, rats (225–250 g) were treated daily between 0900 and 1100 h a single oral gavage of 1 ml/kg body weight (bw) of either vehicle control + Tween-20) or different doses of pentadecafluorooctanoic acid (perfluor-noate; PFOA, purity 95%, CAS Number 335-67-1, Wako Chemicals, n). A similar study on PFOS used a dose of 5 mg/kg, bw (Hu *et al.*, 2005a). is study, 1, 3, 5, 10, 15 mg/kg were used to allow an investigation of the t of PFOA over a wider range of doses and, at the same time, allow a parison with the study on PFOS. Six male rats were randomly assigned to group. Rats in each group were dosed daily with 1, 3, 5, 10, or 15 mg A/kg, bw, daily for 21 days. This experiment was conducted according to uidelines for animal experiments of the National Institute of Animal h, Tsukuba, Japan. At the end of exposure, rats were anesthetized with m pentabarbital and exsanguinated via the abdominal aorta. The liver was ved and portions were immediately frozen in liquid nitrogen and stored 0°C until RNA isolation.

NA extraction. Total RNA was isolated from livers with Trizol Reagent rogen, CA) using manufacturer recommended procedures. The reason for ing liver as the target organ was that perfluorinated fatty acid (PFFAs) y accumulated in blood and liver; the major target organ for PFFAs is med to be liver (Giesy and Kannan, 2001). In addition, a similar study on fects of PFOS on gene expression in the rat had been conducted using total RNA (Hu *et al.*, 2005a). The ratio of the optical densities from RNA es measured at 260 and 280 nm was used to evaluate nucleic acid purity tal RNA concentrations were determined by the absorbance at 260 nm. uality of total RNA was estimated based on the integrity of 28S and 18S . rRNA was separated using 1% agarose gel electrophoresis, good RNA y was indicated by the 28s rRNA banding twice the intensity of the 18s without significant smearing of the rRNA bands. Samples of total RNA from the three rats exposed to the same dose of PFOA were pooled for subsequent use in the GeneChip analysis. Prior to GeneChip analysis, the pooled total RNA samples were purified using the RNeasy Total RNA Mini Kit (Qiagen, Valencia, CA) using manufacturer recommended procedures.

Microarray analysis. Single and double stranded cDNA was synthesized from total RNA samples using SuperScript II (Invitrogen, CA). High-quality total RNA (16 μg) was used as the starting material and 2 μl of 50 μmol T7-Oligo(dT)$_{24}$ Primer (5′-GGCCAGTGAATTGTAATACGACTCACTATAGG-GAGGCGG-(dT)$_{24}$ – 3′; Operon, HPLC purified DNA) was used to prime the reaction. After double stranded cDNA clean up and quality check, an *in vitro* transcription reaction was conducted with the Enzo RNA Transcript Labeling Kit (Affymetrix, Santa Clara, CA) to produce biotin-labeled cRNA from the cDNA. The cRNA was then purified with the RNeasy Mini Kit (Qiagen, Valencia, CA) and fragmented for hybridization analysis. Fifteen microgram aliquants of the fragmented cRNA were hybridized with the Rat Genome 230 2.0 array (Affymetrix, Santa Clara, CA) in hybridization cocktail (0.5 mg/ml cRNA, 50 pM control oligonucleotide B2, 1.5 pM bioB, 5 pM bioC, 25 pM bioD, 100 pM cre, 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 100m M MES, 1 M Na+ 20 mM EDTA, 0.01% Tween 20). Hybridization was allowed to proceed overnight (16 h) at 45°C, followed by washing and staining with streptavidin-phycoerythrin (SAPE, Molecular Probes, Eugene, OR). Hybridization assay procedures including preparation of solutions were carried out as described in the Affymetrix GeneChip Expression Analysis Technical Manual. The distribution of fluorescent material on the array was obtained using G2500A GeneArray Scanner (Affymetrix, Santa Clara, CA). Microarray Suite (MAS) version 5.0 and GeneChip Operating Software (GCOS) supplied by Affymetrix was used to perform gene expression analysis.

Scatter plot, gene tree, and condition tree. Scatter plots were used to visually examine the expression level of genes between the control and PFOA exposed rats. Hierarchical dendrograms were drawn with the Gene tree algorithm of GeneSpring 7 (Silicongenetics, CA). It was similar to phylogenic dendrogram, which was created by clustering the genes according to their expression to the response towards the toxicants. Genes sharing similar expression profiles tended to be clustered together, and the location of a branch containing the genes can be considered a measure of how similar the gene expression was. Condition trees are similar to gene trees, in that they examine the relationships of the expression levels among treatments. Genes were selected for the construction of gene tree if the expression of the gene was two-fold greater or less in the treatments, relative to that in the control (vehicle-exposed) individuals. The horizontal axis shows the clustering of the genes according to their expression profile in the treatment; while the vertical axis showed the clustering according to their expression across treatments.

Pathway analysis. GeneSpring was used to map genes and their expression values on graphical representations of metabolic pathways with reference to the Kyoto Encyclopedia of Genes and Genomes (www.genome.ad.jp/kegg) and GenMAPP organization (www.genmapp.org). A list of pathways affected, the list of genes affected and the *p*-value of genes overlapping with pathway values were created.

Statistical analysis. GeneChip analysis generates very large data sets, it is therefore necessary to evaluate the validity of the data. For the present GeneChip probe array study, the data for each genes represents data from 11–20 probe pairs each approximately 25 bp in length. For each probe pair, one probe is a perfect match while the other has a single mismatch at nucleotide 13. The mismatch probe works as an internal control to evaluate the cross hybridizations between closely related target sequences. The overall target-specific intensity was obtained by the difference between the intensity of perfect match and the mismatch probes. The One-Sided Wilcoxon's Signed Rank test was employed to generate the Detection *p*-value. If the overall intensity of perfect match were much larger than that of mismatch, the detection *p*-value would be small. The probe set would be regarded as present if the *p*-value was less than 0.04 and if the *p*-value was larger than 0.06, the probe set would be regarded as absent.

The GeneChip probe array system only allows comparison of one treatment hybridizing with the probe set. In a Comparison Analysis, two samples were hybridized to two GeneChip probe arrays of the same type, they were compared against each other in order to detect and quantify changes in gene expression. One GeneChip was for baseline (control) and the other was for the experiment (treatment). Prior to performing data comparison, the data were scaled with the rat230_2norm.msk to 2000 in order to correct for variations in overall intensity and for heterogeneity among the GeneChip probe arrays. Two sets of algorithms were generated and they were used to generate change significance and change quantity metrics for every probe set using Microarray Suite (MAS) version 5.0 (Affymetrix, CA). The change algorithm generated a Change p-value and an associated fold-change value. The second algorithm gave a quantitative estimate of the change in gene expression in the form of Signal Log Ratio. In the present study, the level of gene expression can be regarded as increased if its Change p-value was less than 0.0025 and the gene expression would be considered to be decreased if its Change p-value was greater than 0.9975. This method has been used by other investigators (Hu *et al.*, 2005a). Fold change could be calculated with the following formula: Fold change = $2^{(\text{signal log ratio})}$.

RESULTS

The Rat Genome 230 2.0 array is spotted with 31,042 gene probes made of 25-mer single strand oligonucleotides. In the present study, six chips were used. These included one solvent control and five concentrations of PFOA (1, 3, 5, 10, and 15 mg PFOA/kg, bw/day). Comparison analyses of the expression profiles were performed between the control rats and PFOA-treated rats from the GeneChip data. Over 500 genes whose expression was significantly ($p < 0.0025$) altered by at least two-fold after exposure to PFOA at 1 mg/kg, and at 10 mg/kg PFOA the expression of the greatest number of genes (over 800) was altered (Table 1). The use of two-fold cut-off for significance is conventionally used in other similar studies (Barrans *et al.*, 2001; Kume *et al.*, 2005).

Scatter plots were constructed by comparing a composite sample from PFOA-treated rats with that of a composite sample from unexposed (control) rats (Fig. 1). The vertical position of each gene represents its expression level in the treatment of PFOA at different concentrations, and the horizontal position represents its control condition. Those genes above the diagonal (1:1 regression line) were considered to be induced by PFOA exposure while genes below the diagonal were considered to be suppressed. The number of genes deviating from the 1:1 regression line increased as a function of PFOA dose.

TABLE 1
Number of Genes Whose Expression Changed Significantly ($p < 0.0025$) by PFOA

	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg
Up-regulated	199	354	296	488	377
Down-regulated	302	190	220	325	290
Total	501	544	516	813	667

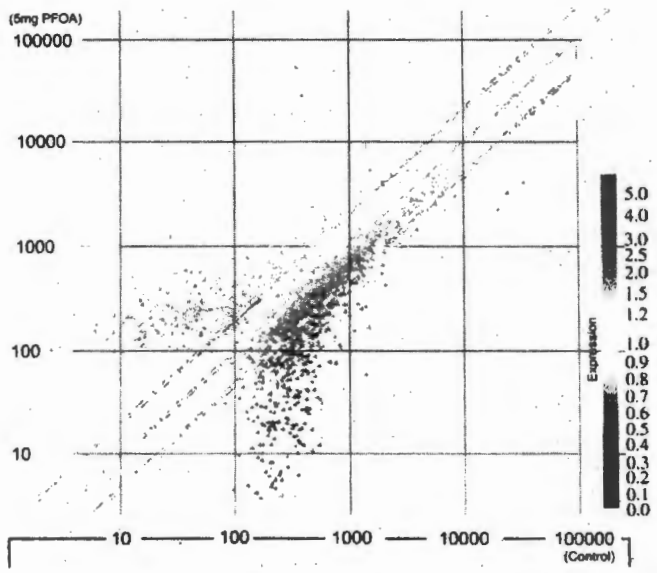
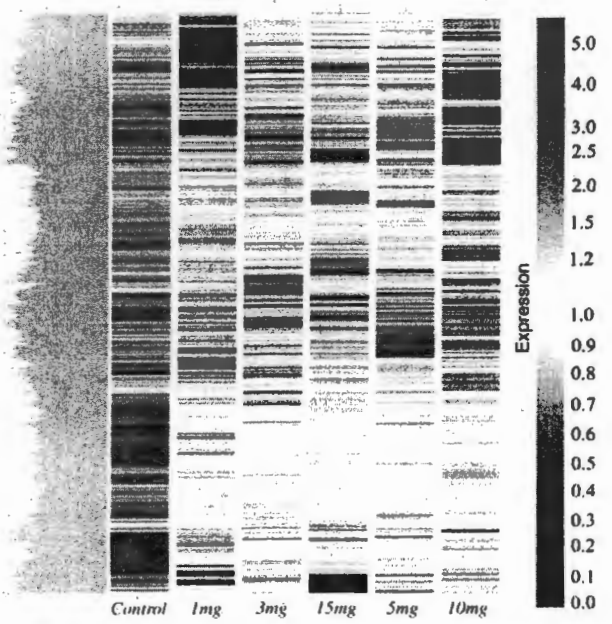


FIG. 1. Scatter plot of gene expression comparisons in rat liver exposure to PFOA. Each point represents a single gene or EST; the diagonal line represents a 1:1 regression and outer lines represent the 95% confidence interval. x-axis: control, y-axis: 5mg PFOA/kg, bw/day treated.

Gene tree analysis created several clusters among the PFOA doses. However, the expression profile of the genes in each cluster was different among treatments. This might be due to different toxico-kinetic mechanisms of different concentrations of PFOA on gene expression. The gene tree analysis classified the patterns of relative responses for all doses relative to the control (Fig. 2). The greatest distance of the node was between the control and the 10 mg PFOA/kg, bw/day exposure. Among the treatment groups, 3 mg and 15 mg PFOA/kg, bw/day were in the same node and this sub-branch was under the node of 5 mg PFOA/kg, bw/day.

The genes altered by the treatment of PFOA of different concentrations could be divided into several large functional categories according to the annotation information and protein information from Protein Knowledgebase provided by Swiss Institute of Bioinformatics (Swiss-Prot) and their biological processes. The genes for which the annotation was unclear such as expressed sequence tags (ESTs) were excluded from the table and when the genes were not fitted into the selected categories, they were regarded as others (Tables 2 and 3). Among the samples from PFOA-treated rats, the largest categories of genes induced were involved in transport and metabolisms of fatty acids and lipids. Other significantly induced genes were involved in cell communication, adhesion, growth, apoptosis, regulation of hormone, proteolysis and peptidolysis and signal transduction. The largest groups of genes suppressed were related to transport, inflammation and immune response and cell adhesion. Several genes involved in apoptosis, regulation of hormone, metabolisms, and G-protein coupled receptor protein signaling pathway were suppressed significantly.



IG. 2. Gene tree dendrogram comparison of all PFOA-altered groups vehicle control exposure.

total of 113 genes and 56 genes were found to be up- ılated and down-regulated, respectively, in at least three of PFOA doses. Among the genes that showed consistent rnations of gene expression in all treatments, 106 were up- ılated while 38 genes were down-regulated. The genes for ch annotation was unclear (ESTs, etc.) were excluded from table (Table 4). The largest grouping of genes up-regulated PFOA exposure were for gene products involved in transport metabolism of lipids, particularly fatty acids. Besides, sev- genes involved in apoptosis; cell communication, adhesion, vth and cycle; signal transduction; regulation of hormone; proteolysis and peptidolysis were also induced significantly. er genes involved in apoptosis, cell adhesion, metabolisms, sport and signal transduction were suppressed significantly. ıthway analysis was conducted by overlapping the genes the KEGG pathways in the Kyoto Encyclopedia of Genes Genomes (www.genome.ad.jp/keg) and GenMAPP orga- tion (www.genmapp.org). The number of common genes each pathway and the random overlap p-value of the gene ıgainst pathway were obtained. KEGG pathways of fatty synthesis (six genes), fatty acid degradation (nine genes), mitochondrial fatty acid β-oxidation (seven genes) were ed significantly ($p < 0.0025$) by exposure of PFOA. Cho- rol synthesis (four genes) and Krebs-TCA Cycle (three s) appeared to be altered but the effect was not statistically ficant.

TABLE 2
Summary of the Up-Regulated genes in Different Concentrations of PFOA According to their Biological Functions

Functional categories of gene	Number of annotated genes				
	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg
Apoptosis	4	6	6	7	5
Cell					
Adhesion	2	2	2	2	3
Communication	0	1	0	1	
Cycle	1	2	1	2	3
Growth	4	5	3	3	5
Proliferation	1	1	1	2	1
Cytoskeleton organization and biogenesis	1	1	1	1	1
Subtotal	9	12	8	11	13
Cytochrome					
Metabolism	4	3	4	4	4
Alcohol	0	2	2	1	2
Amino acids/protein	0	1		1	0
Carbohydrates	5	6	6	6	6
Fatty acids/lipids	19	23	23	25	28
Others	8	9	10	15	12
Subtotal	32	41	41	48	48
Inflammation and immune	1	0	1	2	2
Proteolysis and peptidolysis	2	2	2	1	2
Regulatory of hormone	4	2	4	4	5
Signal transduction	4	5	4	8	6
Stress	2	1	1	3	2
Transport					
Amino acids/protein	1	1	1	2	1
Cholesterol	1	1	1	1	1
Electron	2	2	2	3	2
Fatty acids/lipids	2	3	3	4	5
Ion	2	5	3	4	2
Water	0	1	0	1	2
Others	5	7	5	8	7
Subtotal	13	20	15	23	20
Others	11	19	17	31	27
Total	86	111	103	142	134

The gene expression profiles for rats exposed to 5mg PFOA/kg, bw/day were compared with those reported for rats exposed to PFOS (Hu *et al.*, 2005a) (Tables 5 and 6). Of the 23 genes that were up-regulated by PFOS, 12 genes were also found up-regulated by PFOA. However, seven of the genes were unchanged and four could not be found in the present study. Of the 19 genes for which expression was suppressed by PFOS, only one gene was found to be suppressed and all others but one were not affected by exposure to PFOA.

DISCUSSION

The results of the present study provide insight into how gene expression in livers of rats responded to treatment with several doses of PFOA. Gene expression data was useful in identifying

TABLE 3
Summary of the Down-regulated Gene in Different Concentrations of PFOA According to their Biological Functions

Functional categories of gene	Number of annotated genes				
	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg
Apoptosis	2	2	4	2	2
Cell					
Adhesion	9	4	3	4	3
Communication	1	0	0	0	0
Cycle	3	0		0	1
Growth	1	2	1	1	1
Proliferation	0	2	2	3	2
Cytoskeleton organization and biogenesis	0	0	0	0	0
Subtotal	14	8	6	8	7
Cytochrome	0	0	0	0	0
G-protein coupled receptor protein signaling pathway	12	2	2	11	3
Metabolism					
Amino acids/protein	3	2	4	3	4
Fatty acids/lipids	0	1	0	0	0
Steroid	1	2	2	2	1
Others	7	7	6	9	8
Subtotal	11	12	12	14	13
Inflammation and immune	10	9	6	12	10
Proteolysis and peptidolysis	0	1	1	1	3
Regulatory of hormone	0	0	0	1	0
Signal transduction	4	2	3	2	4
Stress	1	2	1	5	4
Transport					
Amino acids/protein	1	2	0	0	0
Cholesterol	0	1	1	1	1
Oxygen	1	4	0	2	0
Electron	2	5	2	4	3
Fatty acids/lipids	0	4	2	4	3
Ion	8	3	2	12	8
Water	0	1	1	1	2
Others	2	3	4	3	5
Subtotal	14	23	12	27	22
Others	40	16	21	28	28
Total	108	77	68	111	96

the genes involved in the different biochemical pathways which were affected, however, it should not be regarded as a dose-response relationship because the degree of the alternation in gene expression was not solely due to the administered concentration of PFOA. To interpret the potential for effects *in vivo*, other parameters, such as the accumulation of PFOA in the target organ, should be considered. Alteration of the expression of a particular gene does not necessarily mean that a particular protein or biochemical pathway would be affected *in vivo*. However, alteration of a group of genes involved in a particular biochemical pathway would provide strong evidence that PFOA may affect that particular biochemical pathway. Confirmation or isolation of a group of particular proteins or assessment of metabolite flow through the entire target pathway would be the next step in further study and it was not included in the present study.

The number of the genes induced or suppressed was directly proportional to the dose over the range of 1 to 10 mg PFOA/kg, bw/day. However, it was found that the levels of expression of certain genes were different when compared among the concentrations of PFOA. For instance, Resp18, primarily expressed in neural and endocrine tissues, was induced at 1 mg/kg PFOA, was not induced at 3 mg PFOA/kg, bw/day, but was induced again in 5, 10, and 15 mg PFOA/kg, bw/day. In order to understand why a gene was expressed differently at different concentrations of PFOA, the linkage and the pattern of related genes was investigated. However, the linkages of genes were still unclear and it was beyond the scope of the present study to investigate specific pathways in detail. The results of the GeneChip study can, however, be used to design more targeted *in vivo* studies.

Since the results showed that different doses of PFOA resulted in different gene expression profiles, genes which were up- or down-regulated in all concentrations were selected and analyzed in order to understand the effects of different PFOA doses. Overall, our results showed that a large number of genes associated with lipid or fatty acid metabolism were altered by PFOA and some of the genes were linked with pathways of fatty acid degradation and mitochondrial fatty acid β-oxidation in all concentrations of PFOA treated rats. Indeed, similar effects causing interference of fatty acid metabolism had also been found in other studies (Haughom and Spydevold, 1992; Kudo *et al.*, 1999, 2000).

PFOA affected mitochondrial fatty acid β-oxidation, fatty acid synthesis and degradation. The genes coding for the enzymes involved in these mechanisms were generally not all induced significantly at the concentrations of PFOA used, except for the genes responsible for the unsaturated fatty acid metabolism (Tables 7 and 8) and for the transfer of fatty acids for oxidation. Specifically, Cpt1b, Cpt2, and Slc25a20 were induced significantly for all PFOA concentrations. Cpt1b catalyzes the transfer of long chain fatty acids to carnitine for translocation across the mitochondrial inner membrane; Cpt2 coding for the inner mitochondrial membrane protein that converted acylcarnitine to acyl-CoA for further fatty acid metabolism and slc25a20 was a carnitine carrier protein which was a component of the mitochondrial inner membrane and transferred fatty acylcarnitines into the mitochondria. The entrance of fatty acids into the mitochondria requires the activation by acyl-CoA synthetase, however, the genes for acyl-CoA synthetase were not induced significantly, and this suggests that PFOA does not undergo activation by acyl-CoA synthetase. The observation of up-regulated Cpt1b, Cpt2, and Slc25a20 implies that there was an increase in the transfer of activated fatty acids or PFOA across the membrane of the mitochondria, as PFOA was not metabolized (Kudo *et al.*, 2001; Vanden Heuvel *et al.*, 1991), the genes coding for the enzymes for the fatty acid β-oxidation were not significantly

TABLE 4
The Fold Change of Gene Expression under the Exposure of PFOA

ession no.	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg	Gene name	Gene symbol	Group
regulated								
M_053736	2.46	4.00	5.28	6.50	6.06	Caspase 11	Casp4	Apoptosis
M_057108	2.64	2.14	2.83	2.83	3.48	Serine (or cysteine) proteinase inhibitor, clade B, member 5	Serpinb5	Apoptosis
M_053618	2.83	4.00	4.29	4.29	4.00	Bardet-Biedl syndrome 2 (human)	Bbs2	Cell adhesion
F051335	2.14	3.03	2.14	2.83	4.29	Reticulon 4	Rtn4	Cell growth (neurite)
J242963	2.00	2.83	2.00	3.03	3.03	Reticulon 4	Rtn4	Cell growth (neurite)
30789	3.73	2.64	3.73	2.83	4.29	Upregulated by 1,25-dihydroxyvitamin D-3	Txnip	Cell proliferation
M_012753	2.64	5.28	5.28	7.46	6.96	Cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	Cytochrome
I454613	8.00	6.50	9.19	7.46	9.85	Cytochrome P450, 2b19	Cyp2b15	Cytochrome/metabolism—fatty acid
M_023025	2.83	3.25	3.48	3.73	3.73	CYP2J4	Cyp2j4	Cytochrome/metabolism—fatty acid
M_017083	8.57	14.93	8.57	14.93	21.11	Myosin 5B	Myo5b	Cytoskeleton organization and biogenesis
)7202	16.00	22.63	68.59	73.52	78.79	Asparagine synthetase	Asns	Metabolism
M_022936	4.92	5.28	4.00	6.06	5.28	Epoxide hydrolase 2, cytoplasmic	Ephx2	Metabolism
M_012600	2.00	4.59	4.92	6.50	6.50	Malic enzyme 1	Me1	Metabolism
M_024398	2.46	3.03	2.46	2.46	2.64	Aconitase 2, mitochondrial	Aco2	Metabolism—carbohydrates
30596	14.93	64.00	34.30	55.72	36.76	Malic enzyme 1	Me1	Metabolism—carbohydrates
M_053551	3.25	4.29	4.59	5.28	5.66	Pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	Metabolism—carbohydrates
M_130747	3.03	2.64	2.46	2.46	3.25	Cytoplasmic acetyl-CoA hydrolase	rACH	Metabolism—carbohydrates
M_022215	2.83	3.25	3.25	4.00	4.29	Glycerol-3-phosphate dehydrogenase 1 (soluble)	Gpd1	Metabolism—carbohydrates (testis)
M_022407	4.29	4.29	5.28	5.28	5.66	Aldehyde dehydrogenase family 1, member A1	Aldh1a1	Metabolism—fatty acid
3072411	7.46	7.46	12.13	9.19	11.31	cd36 Antigen	Cd36	Metabolism—fatty acid
M_133586	4.59	5.28	5.28	4.92	6.96	Carboxylesterase 2 (intestine, liver)	Ces2	Metabolism—fatty acid
M_013200	4.29	12.13	10.56	18.38	12.13	Carnitine palmitoyltransferase 1b	Cpt1b	Metabolism—fatty acid
M_012930	2.30	2.46	2.30	2.46	3.03	Carnitine palmitoyltransferase 2	Cpt2	Metabolism—fatty acid
M_017306	2.46	2.00	2.83	2.30	3.25	Dodecenoyl-coenzyme A delta isomerase	Dci	Metabolism—fatty acid
M_022594	3.03	2.64	3.25	3.03	3.73	enoyl Coenzyme A hydratase 1, peroxisomal	Ech1	Metabolism—fatty acid
M_133606	6.50	5.28	7.46	6.96	8.57	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	Ehhadh	Metabolism—fatty acid
116152	42.22	24.25	73.52	48.50	119.43	Fatty acid elongase 2	rELO2	Metabolism—fatty acid
M_012489	5.66	4.29	6.06	4.59	6.96	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	Acaa1	Metabolism—fatty acid (peroxisomal)
3921	2.64	2.14	3.03	2.64	3.48	acetyl-Coenzyme A acetyltransferase 1	Acat1	Metabolism—fatty acid (peroxisomal)
9333	24.25	32.00	25.99	36.76	36.76	Mitochondrial acyl-CoA thioesterase 1	Mte1	Metabolism—fatty acid (peroxisomal)

TABLE 4—*Continued*

Accession no.	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg	Gene name	Gene symbol	Group
Y09333	4.92	6.96	5.66	9.19	8.00	Mitochondrial acyl-CoA thioesterase 1	Mte1	Metabolism—fatty acid (peroxisomal)
NM_130756	2.14	3.03	2.64	2.83	3.73	4,8-Dimethylnonanoyl-CoA thioesterase	Pte1	Metabolism—fatty acid (peroxisomal)
NM_031315	207.94	222.86	256.00	362.04	315.17	cytosolic acyl-CoA thioesterase 1	Cte1	Metabolism—lipid
J02585	2.64	4.92	5.28	5.28	8.00	stearoyl-Coenzyme A desaturase 1	Scd1	Metabolism—fatty acid
NM_134395	2.14	2.46	2.30	2.46	2.30	P11 protein	Cdtw1	Other
AA945082	2.14	2.64	2.30	2.14	3.25	Glutathione-S-transferase, alpha type2	Gsta2	Other
NM_053487	3.03	4.00	5.28	6.50	5.28	Peroxisomal biogenesis factor 11A	Pex11a	Other
NM_012500	2.30	3.48	3.25	3.25	4.59	N-acylaminoacyl-peptide hydrolase	Apeh	Proteolysis and peptidolysis
M27882	5.28	8.00	11.31	12.13	9.85	Serine protease inhibitor, Kazal type 1	Spink1	Regulatory of hormone
M27882	4.29	6.06	8.00	9.19	6.06	Serine protease inhibitor, Kazal type 1	Spink1	Regulatory of hormone
NM_019274	2.14	3.25	2.64	3.25	3.25	Collagen-like tail subunit (single strand of homotrimer) of asymmetric acetylcholinesterase	Colq	Signal transduction
AY081195	3.73	4.00	5.28	4.92	6.06	Monoglyceride lipase	Mgll	Signal transduction
NM_053923	4.00	25.99	21.11	32.00	21.11	Phosphatidylinositol 3-kinase, C2 domain containing, gamma polypeptide	Pik3c2g	Signal transduction
NM_021684	14.93	17.15	34.30	36.76	21.11	Soluble adenylyl cyclase	Sac	Signal transduction (testis)
M63991	2.64	4.92	4.00	6.06	3.25	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	Serpina7	Transport— hormone
NM_019283	2.46	2.64	2.64	4.00	3.48	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	Slc3a2	Transport—amino acid
NM_053502	4.29	3.25	3.48	5.28	6.06	ATP-binding cassette, sub-family G (WHITE), member 1	Abcg1	Transport—cholesterol
NM_016999	3.48	2.46	3.48	2.83	4.29	Cytochrome P450, family 4, subfamily b, polypeptide 1	Cyp4b1	Transport—electron/induce bladder cancer
NM_031561	4.92	6.50	7.46	6.50	7.46	cd36 antigen	Cd36	Transport—fatty acid
J02844	3.25	3.73	6.06	6.96	6.50	Carnitine O-octanoyltransferase	Crot	Transport—fatty acid
M38759	27.86	42.22	59.71	97.01	64.00	Histidine decarboxylase	Hdc	Transport—hormone
NM_019269	3.03	2.46	3.25	4.59	4.59	Solute carrier family 22 (organic cation transporter), member 5	Slc22a5	Transport—ion
J03093	2.30	2.00	2.64	2.30	2.83	Odorant binding protein I f	Obp1f	Transport-/odour
Down-regulated								
NM_012649	−2.14	−4.92	−2.46	−3.48	−3.73	Syndecan 4	Sdc4	Apoptosis
AB030829	−2.14	−2.64	−2.14	−3.03	−2.30	Carbonic anhydrase 3	Ca3	Cell adhesion
NM_017061	−6.96	−9.85	−13.00	−39.40	−12.13	Lysyl oxidase	Lox	Metabolism
BI304009	−9.85	−4.92	−16.00	−3.73	−9.85	Lysyl oxidase	Lox	Metabolism
NM_012737	−2.00	−3.73	−8.00	−10.56	−7.46	Apolipoprotein A-IV	Apoa4	Metabolism—one carbon
NM_022526	−2.30	−5.28	−4.00	−6.96	−4.59	Death-associated protein	Dap	Metabolism—steroid

TABLE 4—*Continued*

ession no.	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg	Gene name	Gene symbol	Group
F249673	−2.14	−2.64	−3.25	−3.73	−3.48	Solute carrier family 38, member 2	Slc38a2	Other
M_017080	−2.14	−3.25	−3.73	−4.92	−4.00	Hydroxysteroid 11-beta dehydrogenase 1	Hsd11b1	Other
M_012695	−2.30	−3.03	−3.48	−3.73	−2.46	Rat senescence marker protein 2A gene, exons 1 and 2	Smp2a	Other
)2422	−2.14	−2.64	−2.83	−6.50	−3.48	Cytochrome P450, family 1, subfamily a, polypeptide 2	Cyp1a2	Signal transduction
M_019344	−3.03	−3.73	−27.86	−24.25	−2.64	Regulator of G-protein signaling 8	Rgs8	Transport—electron. Metabolism—xenobiotic
M_031533	−3.03	−2.64	−4.92	−8192.00	−2.46	UDP glycosyltransferase 2 family, polypeptide B	Ugt2b	Transport—lipid
)2601	−2.46	−2.14	−2.14	−2.83	−3.03	20 alpha-Hydroxysteroid dehydrogenase	LOC171516	Transport—nitrogen

egulated among the concentrations of PFOA. PFOA has a ilar structure to endogenous fatty acids except that fluorine ns replace all the hydrogen atoms linked with carbon atoms ie fatty acids. Therefore, it is possible that PFOA could be guided by the fatty acid metabolism machinery as a sub- e because of its structural similarity to endogenous fatty s, and this effect is similar to those observed for PFOS (Hu *l.*, 2005a). Although the genes coding for the enzymes for acid degradation, β-oxidation and synthesis were not ificantly up-regulated among the concentrations of PFOA, genes coding for the enzymes involved in the metabolism of turated fatty acids were up-regulated. Therefore, there ht have been some interactions between PFOA and the path- s that facilitated the degradation of unsaturated fatty acids. animal cells, mitochondria and peroxisomes can oxidize acids via β-oxidation. There are two major differences een mitochondrial and peroxisomal β-oxidation, in terms s specificity and mechanism (Reddy and Mannaerts, 1994; ders *et al.*, 2001). In mammals, the mitochondria oxidize t, medium, and most long chain fatty acids, while peroxi- es oxidize some long chain and very long chain fatty acids. o *et al.* (2000) demonstrated that PFOA induced peroxi- al β-oxidation in *in vivo* and *in vitro* studies. In the present y, it was found that the genes coding for peroxisomal oxi- on were induced significantly especially at a dose of 10 mg A/kg, bw/day (Fig. 3). Since PFOA is non-biodegradable, ccumulation of PFOA could have resulted in a significant ction of the genes coding for enzymes responsible the adation of fatty acids. Because the expression of catalase not induced by PFOA it is possible that potentially toxic ogen peroxide was produced in peroxisomes from acyl- that could have caused oxidative stress or oxidation age to the proteins and DNA. A similar explanation of the ts of PFOS has been previously hypothesized (Hu *et al.*, a). Furthermore, peroxisomes could also oxidize the side chains of eicosanoids, molecules important in short-range signaling, derived from arachidonic acid. Prostaglandins and leukotrienes are representatives of the family of eicosanoid; hence, they might be oxidized and could not perform their normal functions. The gene coding for one of the enzymes responsible for the metabolism of prostaglandins, hpgd, was significantly up-regulated by 3-fold by the 5 mg PFOA/kg, bw/day dose and 4-fold by the 15 mg PFOA/kg, bw/day dose. This observation suggests increased metabolism of prostaglandins.

Both mitochondrial and peroxisomal isomerases were up-regulated. The genes Dci and Decr1, which are involved the mitochondrial β-oxidation; and the genes Ech1 and Decr2, which are involved in the peroxisomal β-oxidation, were also up-regulated. These isomerases are enzymes involved in the oxidation of the long-chain fatty acids in peroxisomes (Fig. 3). These enzymes, which help to oxidize unsaturated fatty acids through the isomerization of 3-*trans*-, 5-*cis*-dienoyl-CoA to 2-*trans*-, 4-*trans*-dienoyl-CoA were up-regulated by the peroxisome proliferator [clofibrate or di (2-ethylhexyl) phthalate] (FitzPatrick *et al.*, 1995). Up-regulation of these genes implies that there was increased unsaturated fatty acids metabolism. Therefore, there might be some interaction between PFOA and unsaturated fatty acids, or PFOA was mistaken as the substrate for the unsaturated fatty acid β-oxidation in both the mitochondrial and peroxisomal pathways. However, no conclusion could be drawn here because further confirmation of an increased metabolism of unsaturated fatty acids is required.

In the present study, the expression of Cyp4b1 was significantly up-regulated (2.8- to 4.3-fold) among the PFOA treatments (Table 4). Several studies have shown that the induction of the Cyp4b1 gene could be related to bladder cancer in rabbits, mice, and humans (Imaoka *et al.*, 1995, 2000, 2001). Cyp4b1 has an important role in mutagenic activation of procarcinogens in the bladder. Cyp4b1 expression can be induced by benzidine and 2-naphthylamine, which have been

TABLE 5
List of Genes Induced in Rat Treated with 5 mg PFOA/kg, bw/day and PFOS

Accession no.	Gene name	Gene Name	PFOA	PFOS[a]
Genes induced in both treatments of PFOA and PFOS				
AI454613	Cytochrome P450, 2b19	Cyp2b15	9.19	9.09
NM_031315	Cytosolic acyl-CoA thioesterase 1	Cte1	256	90
NM_133586	Carboxylesterase 2 (intestine, liver)	Ces2	5.28	5.89
Y09333	Mitochondrial acyl-CoA thioesterase 1	Mte1	5.66	11
NM_017306	Dodecenoyl-coenzyme A delta isomerase	Dci	2.83	6.02
NM_012489	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	Acaa1	6.06	9.78
NM_133606	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	Ehhadh	7.46	6.5
NM_022594	enoyl Coenzyme A hydratase 1, peroxisomal	Ech1	3.25	5.11
NM_022407	Aldehyde dehydrogenase family 1, member A1	Aldh1a1	5.28	6.04
NM_012753	Cytochrome P450, family 17, subfamily a, polypeptide 1	Cyp17a1	5.28	19.3
NM_019278	Regulated endocrine-specific protein 18	Resp18	4.00	6.44
M38759	Histidine decarboxylase	Hdc	60	12
AF079864	Olfactory receptor gene Olr59	Olr59	3.48	3.96
Genes induced only in PFOS treatment				
D38381	Cytochrome P450, 3a18	Cyp3a18		6.15
NM_031841	Stearoyl-Coenzyme A desaturase 2	Scd2		12.88
NM_019203	Testis specific X-linked gene	Tsx		3.5
U09742	Cytochrome P450, family 3, subfamily a, polypeptide 11	Cyp3a11		5.19
NM_031013	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	Abcc6		5.5
NM_053480	DNA polymerase alpha subunit II	Pola2		13.54
Genes induced only in PFOA treatment				
NM_133420	Cholinergic receptor, nicotinic, alpha polypeptide 2 (neuronal)	Chrna2	5.28	
NM_019269	Solute carrier family 22 (organic cation transporter), member 5	Slc22a5	3.25	
U30789	Upregulated by 1,25-dihydroxyvitamin D-3	Txnip	3.73	
NM_012500	N-acylaminoacyl-peptide hydrolase	Apeh	3.25	
NM_133609	Eukaryotic translation initiation factor 2B, subunit 3 (gamma, 58kD)	Eif2b3	13.00	
BI273703	Peroxisomal biogenesis factor 11A	Pex11a	5.66	
NM_053487	Peroxisomal biogenesis factor 11A	Pex11a	5.28	
NM_012600	Malic enzyme 1	Me1	4.92	
BG378763	Glycerol-3-phosphate dehydrogenase 2	Gpd2	3.48	
NM_013200	Carnitine palmitoyltransferase 1b	Cpt1b	10.56	
AF072411	cd36 antigen	Cd36	12.13	
NM_012598	Lipoprotein lipase	Lpl	6.06	
NM_031987	Carnitine O-octanoyltransferase	Crot	3.25	
J02844	Carnitine O-octanoyltransferase	Crot	6.06	
NM_053502	ATP-binding cassette, sub-family G (WHITE), member 1	Abcg1	3.48	
NM_012904	Annexin A1	Anxa1	3.25	
NM_053618	Bardet-Biedl syndrome 2 (human)	Bbs2	4.29	
U07202	Asparagine synthetase	Asns	68.59	
NM_022936	Epoxide hydrolase 2, cytoplasmic	Ephx2	4.00	
NM_053736	Caspase 11	Casp4	5.28	
NM_130422	Caspase 12	Casp12	3.25	
NM_053551	Pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	4.59	
NM_031147	Cold inducible RNA binding protein	Cirbp	4.92	
NM_053923	Phosphatidylinositol 3-kinase, C2 domain containing, gamma polypeptide	Pik3c2g	21.11	
NM_017083	Myosin 5B	Myo5b	8.57	
AF413572	Protein kinase (cAMP dependent, catalytic) inhibitor beta	Pkib	4.29	
M27882	Serine protease inhibitor, Kazal type 1	Spink1	11.31	
M23995	Aldehyde dehydrogenase family 1, subfamily A4	Aldh1a4	6.96	
NM_053299	Ubiquitin D	Ubd	3.48	
NM_021684	Soluble adenylyl cyclase	Sac	34.30	
NM_022215	Glycerol-3-phosphate dehydrogenase 1 (soluble)	Gpd1	3.25	
J02585	stearoyl-Coenzyme A desaturase 1	Scd1	5.28	
AY081195	Monoglyceride lipase	Mgll	5.28	
M63991	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), mem: 7	Serpina7	4.00	
NM_024390	NAD-dependent 15-hydroxyprostaglandin dehydrogenase	Hpgd	4.00	

TABLE 5—*Continued*

ssion no.	Gene name	Gene Name	PFOA	PFOS[a]
27882	Serine protease inhibitor, Kazal type 1	Spink1	8.00	
M_023025	CYP2J4	Cyp2j4	3.48	
M_024390	NAD-dependent 15-hydroxyprostaglandin dehydrogenase	Hpgd	4.00	
E116152	Fatty acid elongase 2	rELO2	73.52	
1008369	Tumor specific antigen 70 kDa	Loc192276	13.00	

[a]FOS data cited in Hu *et al.*, 2005a.

ıtified as carcinogens that can initiate bladder carcinomas aoka *et al.*, 1997). Caspase-11, a critical activator of several er caspase-genes involved in the cytokine regulation of ptosis, was also induced in PFOA treated animals compared he controls. Likewise, it is noteworthy that a number of es involved in immune function, such as interleukin, roglobulin, and leukocyte activation were down-regulated at least three or four of the PFOA doses. Additionally, pression of G-protein associated signaling pathway genes h as Ghsr, Avpr1a, and Hcrtr2 might affect regulation of d intake and energy homeostasis by affecting mechanisms ieuronal transmission.

FOS, one of the predominant PFCs detected in the ironment and accumulated into biota, is structurally anal- us to of PFOA except for the terminal sulfonic acid group as ıpared to the terminal carboxylate of PFOA and a different ıber of carbon atoms in PFOA, 7 carbon chain attached to terminal carboxylic acid compared to the 8 carbon chain of)S. To determine the similarity of effects on gene expression hese two PFCs, the results of PFOA on gene expression in current study to that of PFOS were compared (Tables 5 and The genes that were up- or down-regulated by PFOA were e different from those for which gene expression was red by PFOS that might be due to the large differences in r physical and chemical properties however it needs further stigations. Cytochrome groups were of interest as they e related to the metabolism of xenobiotics. Up-regulation of 2b15, which is responsible for catalytic reactions involved rug metabolism, was similar for PFOA and PFOS (Table 5). vever, the response of Cyp17a1, which is responsible for oid hydroxylation and comprises a main component of the oidogenic pathway, was up-regulated by PFOS about 4-fold e than by PFOA. Two cytochromes Cyp3a18 and Cyp3a11, ch are responsible for the conversion and the hydroxylation estosterone, were up-regulated by PFOS, but not PFOA. 2j4, which is a putative cytochrome p450 monooxygenase yme, was up-regulated PFOA but not PFOS. To conclude, cytochrome genes induced by PFOA were mainly related to obiotic metabolisms while those of PFOS were related to ı xenobiotic metabolisms as well as steroid metabolisms.

oth PFOS and PFOA up-regulated genes responsible for ymes involved in fatty acid and lipid metabolism which ht be due to their structural similarity to endogenous fatty acids. These compounds may act as structural analogues of endogenous fatty acids and the differences in the gene expression profiles might be due to the different functional groups of PFOA and PFOS, suggesting that further investigation on the toxicity of PFOA is necessary. In this study we observed that PFOA affected both peroxisomal and mitochondrial fatty acids oxidation while PFOS has been reported to mostly affect peroxisomal oxidation (Hu *et al.*, 2005a). The fold change of the enzymes up-regulated by both the 5 mg PFOS/kg, bw/day dose and all doses of PFOA were similar for most of the genes along the peroxisomal fatty acid oxidation pathway except for Acaa1. The fold change of Acaa1 induced by PFOS was approximately twice that of PFOA (Tables 5 and 8).

While there were similarities in modulation of gene expression by PFOS and PFOA, there were also differences. Some of these differences could have been due to different aspects of the design of the two experiments being compared. For instance, the study of PFOS used female rats while that on PFOA used male rats. The reason for choosing male rats was that the elimination rate of PFOA in female was much faster than that of male (Hanhijarvi *et al.*, 1982; Kudo *et al.*, 2002; Vanden Heuvel *et al.*, 1991), the use of male rats will allow this study on PFOA to have more similar toxicokinetics as compared to the previous study on PFOS. Although there was a difference in sex, both PFOA and PFOS induced the genes in the peroxisomal β-oxidation pathway.

PFOA has been shown to interfere with fatty acid metabolism and cholesterol synthesis in the liver (Haughom and Spydevold, 1992). Studies of selected enzymes demonstrated a significant decrease in Hmgcr, acyl coenzyme A (CoA) cholesterol acyltranseferase (ACAT) activities, which were related to a lesser concentration of serum cholesterol after 24 h when rats fed PFOA. In the present study, expression of the gene that codes for Hmgcr activity was significantly down-regulated at doses of 5, 10, and 15 mg PFOA/kg, bw/day. Hmgcr is an enzyme involved in mevalonate synthesis which is a rate limiting enzyme in the cholesterol synthesis pathway. This is consistent with previous studies that have found that alteration in the activity of this gene may contribute to the hypolipidemic effect caused by PFOA. Any effects on the expression of the gene that codes for Hmgcr could affect production of lipoprotein particles because of the reduced synthesis of cholesterol together with the increased oxidation of fatty acids in the liver

TABLE 6
List of Genes Suppressed in Rat Treated with 5 mg PFOA/kg, bw/day and PFOS

Accession no.	Gene title	Gene name	PFOA	PFOS[a]
Genes suppressed in both treatments of PFOA and PFOS				
NM_012737	Apolipoprotein A-IV	—	−7.69	−5.9
Genes suppressed in only PFOS treatment				
AI715202	RT1 class II, locus Bb	RT1-Bb		−16.38
NM_053999	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	Ppp2r2a		−5.81
NM_012942	Cholesterol hydroxylase 7alpha	—		−1.83
NM_012633	Peripherin 1	Prph1		−2.65
NM_017230	Peptidyl arginine deiminase, type III	Padi3		−1.76
AI234969	GATA binding protein 4	Gata4		−5.03
NM_019907	Postsynaptic protein Cript	Cript		−3.26
U21683	Spleen tyrosine kinase	Syk		−2.79
NM_080584	Phosphorylase kinase, gamma 2 (testis)	Phkg2		−3.37
BF419200	CCAAT/enhancer binding protein (C/EBP), delta	Cebpd		−0.51
NM_012505	ATPase, Na+K+ transporting, alpha 2	Atp1a2		−11.14
NM_017335	GABA transporter	Slc6a12		−3.25
NM_013027	Selenoprotein W, muscle 1	Sepw1		−3.12
U35098	Complexin 1	Cplx1		−0.16
Genes suppressed only in PFOA treatment				
BE349669	Caveolin 2	Cav2	−7.46	
AI406385	Caveolin 2	Cav2	−17.15	
BE349669	Caveolin 2	Cav2	−4.59	
NM_017080	Hydroxysteroid 11-beta dehydrogenase 1	Hsd11b1	−3.73	
NM_019340	Regulator of G-protein signalling 3	Rgs3	−4.29	
AI549431	Epidermal growth factor receptor	Egfr	−3.25	
NM_031560	Cathepsin K	Ctsk	−4.92	
NM_017061	Lysyl oxidase	Lox	−13.00	
BI304009	Lysyl oxidase	Lox	−16.00	
NM_130403	Protein phosphatase 1, regulatory (inhibitor) subunit 14A	Ppp1r14a	−9.85	
NM_139258	Bcl2 modifying factor	Bmf	−9.19	
AI169634	X transporter protein 3	Xtrp3	−18.38	
D16438	Opioid receptor-like	Oprl	−32.00	
NM_031533	UDP glycosyltransferase 2 family, polypeptide B	Ugt2b	−4.92	
L25527	Selectin, endothelial cell	Sele	−42.22	
NM_021836	Jun-B oncogene	Junb	−4.00	
BG378312	Chondroitin sulfate proteoglycan 6	Cspg6	−12.13	
NM_053372	Secretory leukocyte protease inhibitor	Slpi	−3.25	
NM_022526	Death-associated protein	Dap	−4.00	
AF249673	Solute carrier family 38, member 2	Slc38a2	−3.25	
BF283692	Keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)	Krt5	−4.92	
AI070976	Low density lipoprotein receptor-related protein 4	Lrp4	−9.85	
AI137995	Sodium channel, voltage-gated, type IV, beta	Scn4b	−6.06	
AI412189	Partial mRNA for immunoglobulin alpha heavy chain (partial), complete constant region	—	−4.29	
AW525176	Filamin A interacting protein 1	Filip1	−16.00	
AI045191	Complement component 6	C6	−4.00	
AA819776	Heat shock protein 1, alpha	Hspca	−6.96	
NM_012695	Rat senescence marker protein 2A gene, exons 1 and 2	Smp2a	−3.48	
AI176519	Immediate early response 3	Ier3	−3.48	
AW530219	Ryanodine receptor 1 (skeletal)	Ryr1	−4.00	
BE117439	Phosphatidylinositol 3-kinase, catalytic, alpha polypeptide	Pik3ca	−5.66	
	Genes differentially expressed in PFOA and PFOS treaments			
AF014009	Peroxiredoxin 6	Prdx6	1.15	−2.45

[a]PFOS data cited in Hu *et al.*, 2005a.

TABLE 7
List of Genes Responsible for the Transport in Fatty Acid Metabolisms in Rat Treated with PFOA

ession no.	Gene title	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg
y acid synthesis						
)2585	stearoyl-Coenzyme A desaturase 1	2.64	4.92	5.28	5.28	8.00
M_031841	stearoyl-Coenzyme A desaturase 2	6.50	5.66	3.48	11.31	4.92
›chondrial fatty acid β-oxidation/fatty acid degradation						
M_017306	dodecenoyl-Coenzyme A delta isomerase	2.46	2.00	2.83	2.30	3.25
M_057197	2,4-dienoyl CoA reductase 1, mitochondrial	1.74	1.41	1.62	1.74	2.00
M_053965	Solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	1.87	2.00	2.30	2.00	2.46
M_012930	Carnitine palmitoyltransferase 2	2.30	2.46	2.30	2.46	3.03
M_013200	Carnitine palmitoyltransferase 1b	4.29	12.13	10.56	18.38	12.13

ughom and Spydevold, 1992). Cholesterol is an important ıponent of the cell membrane; it stablizes the membrane's dity over a bigger temperature interval. Since down-ılation of this gene with a concomitant decrease in Hmgcr vity would result in less cholesterol synthesis, it might alter cell membrane fludity, an effect that has also been observed ›e caused by PFOS (Hu *et al.*, 2003). It may even be othesized that increases in membrane fulidity caused by osure to PFOS result in decreased synthesis of cholesterol down-regulation of Hmgcr. Another gene, Abcg1, which is ›onsible for cholesterol transport, was significantly up-ılated (Table 4). Since cholesterol production has been ıd to be decreased by exposure to both PFOS and PFOA, efflux of excess cholesterol from macrophages would have helped maintain cholesterol homeostasis. Obviously, gene expression studies can not be used to directly investigate effects on cell membrane fluidity. However, recent studies have shown that PFOS can be incorporated into cell membranes resulting in altered cell membrane fluidity and membrane potential (Hu *et al.*, 2003). PFOS also affected gap junction intercellular communication (Hu *et al.*, 2002). Similar effects of PFOA to those of PFOS on membrane fluidity can be postulated, but further research would be necessary to demonstrate this phenomenon. PFOA both up- and down-regulated gene expression that are related to signal transduction and G-protein coupled receptors. This is consistent with the

TABLE 8
List of Genes in All Concentrations Responsible for the Peroxisomal and Mitochondrial Fatty Acid β-Oxidation

		PFOA					PFOS[a]
ssion no.	Gene name	1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg	15 mg/kg	5 mg/kg
›isomal fatty acid β-oxidation							
90101	acyl-CoA synthetase	1.41	—	1.41	1.52	1.62	1.5
1_17340	acyl-CoA oxidase	2.3	1.62	2.64	2.14	2.83	2
044574	2.4 Dienoyl-Coenzyme A reductase 2	2	1.87	2	1.62	2.64	—
1_022954	enoyl Coenzyme A hydratase 1	3.03	2.64	3.25	3.03	3.73	—
1_133606	enoyl Coenzyme A hydratase	6.5	5.28	7.46	6.96	8.57	6.5
1_0133606	3-hydroxyacyl Coenzyme A dehydrogenase	6.5	5.28	7.46	6.96	8.57	6.5
1_012489	3-oxoacyl-Coenzyme A thiolase	5.66	4.29	6.06	4.29	6.96	10
hondrial fatty acid β-oxidation							
1_013200	Carnitine palmitoyltransferase 1b	4.29	12.13	10.56	18.38	12.13	1
1_012930	Carnitine palmitoyltransferase 2	2.3	2.46	2.3	2.46	3.03	1.2
1_016986	acetyl-Coenzyme A dehydrogenase	1.41	—	1.23	1.23	1.32	1.1
1_057197	2,4-dienyl-Coenzyme A dehydorgenase	1.74	1.41	1.62	1.74	2.00	—
1_017306	dodecenoyl-CoA delta isomerase	2.46	2	2.83	2.3	3.25	—
800240	enoyl-Coenzyme hydratase	1.62	1.74	1.62	1.74	1.87	1.2
800240	hydroxyacyl-Coenzyme A dehydrogenase	1.62	1.74	1.62	1.74	1.87	1.2
1_133618	3-ketoacyl-Coenzyme A thiolase	1.87	2	1.87	2.14	2.3	1.5

[a] OS data cited in Hu *et al.*, 2005a.

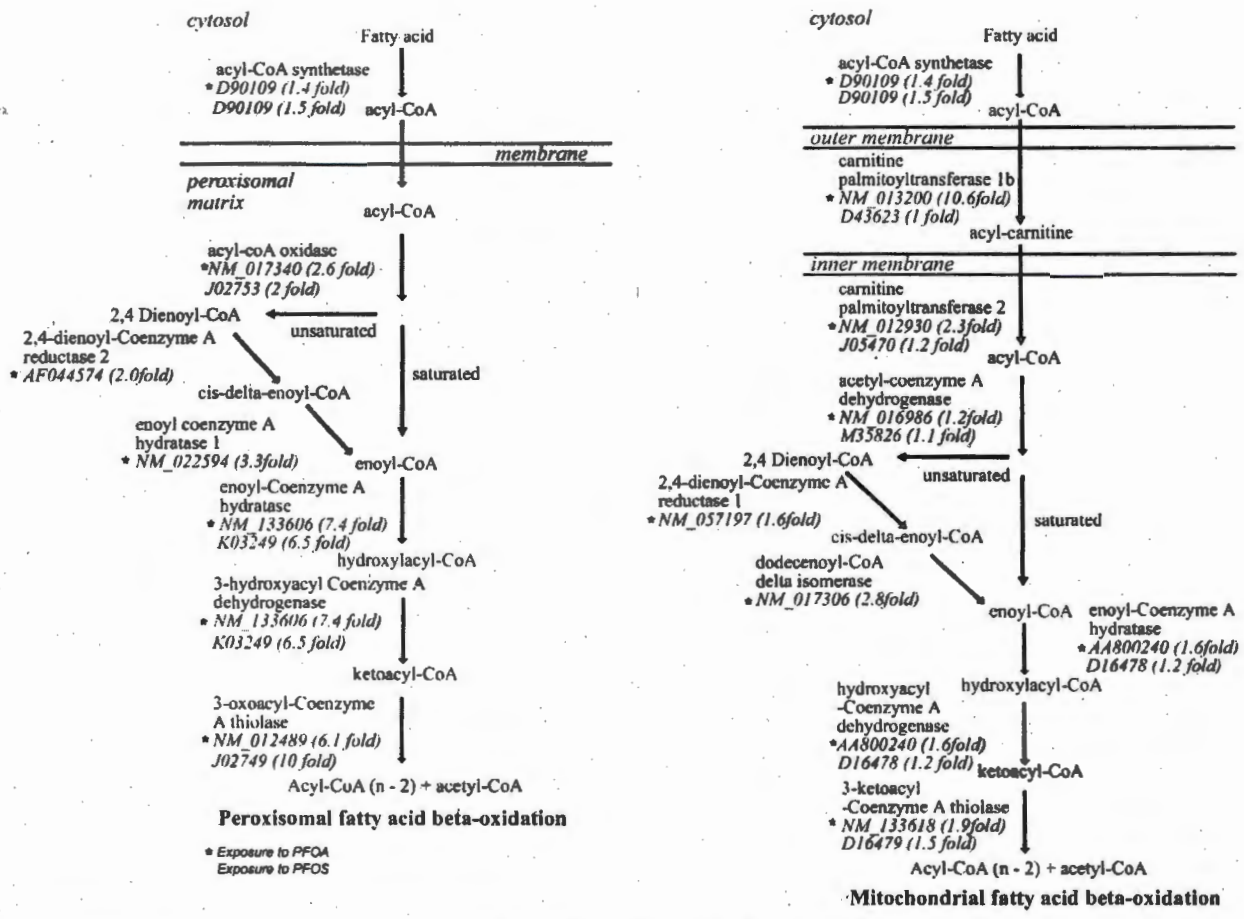


FIG. 3. Diagram of pathways for peroxisomal and mitochondria fatty acid β-oxidation and the relative induction of the enzymes due to PFOA exposure to rats; PFOS data were cited in Hu *et al.*, 2005a.

observation that PFOA can inhibit gap junction intercellular communication (Upham *et al.*, 1998).

It has been suggested that both slc22a7 (Oat 2—older name) and slc22a8 (Oat3—older name) are responsible for the urinary elimination of PFOA (Kudo *et al.*, 2002). In previous studies, neither slc22a7 nor slc22a8 were down-regulated by any of the doses of PFOS, however both, slc21a1 (Oatp1) and slc21a5 (Oatp2) were down-regulated by some PFOA doses. The genes slc22a7, slc22a8, slc21a4, slc21a1, and slc21a5 are involved in transport of organic anions and they might also be involved in the urinary elimination of PFOA. Since all of these genes were suppressed, it would be expected that exposure to PFOA would result in a negative feedback that would inhibit elimination of PFOA in the rat.

Here we have reported, for the first time, information on the gene expression profiles of rats receiving different doses of PFOA. These results can be used to understand the biochemical processes affected by PFOA. PFOA enhanced both peroxisomal and mitochondrial fatty acid β-oxidation and the peroxisomal β-oxidation might create oxidative stress on DNA and protein. Besides, the reduction of cholesterol synthesis observed in *in vitro* studies would be consistent with the down-regulation of the gene Hmgcr observed in this study. Furthermore, this study suggests that the suppression of organic anion transport genes may explain the delayed urinary clearance of PFOA. There were a number of genes related to tumor progression and inflammation affected by exposure to PFOA, which suggests that exposure to PFOA might enhance the risk of cancer. The outcome of gene data comparison between PFOA and PFOS suggests that the responses in gene expression are similar, but not identical for the two substances. Furthermore the similarities in the effects on lipid metabolism suggest similar modes of action, but the exact mechanisms of the effects may be different and/or may be affected to different degrees by PFOA and PFOS. The responses of genes in the critical pathways are sufficient to consider the development of relative potency factors for PFOS and PFOA to be used in risk assessments. However, differences in the toxicokinetics of PFOA and PFOS would need to be considered if the effects of the two compounds on a single pathway were considered.

ACKNOWLEDGMENTS

This research work was partially supported from the Japanese Ministry of vironment under the Global Environment Conservation Research Fund 04–2008) to K.S.G. and by a CERG grant (CityU1401/05M) from the Hong ng Research Grants Council awarded to P.K.S.L. The authors also thank H. Hoshiba and animal care members of NIAH for their invaluable istance during the study. Conflict of interest: none declared.

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